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Heparin-Binding Domain of Human Fibronectin Binds HIV-1 gp120/160 and Reduces Virus Infectivity

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In vitro experiments indicate that components of the host present in body fluids may prevent the attachment of human immunodeficiency virus type 1 (HIV-1) to target cells. Fibronectin (Fn), a dimeric 440-kDa extracellular matrix adhesion protein, is secreted by mesenchymal cells and assembled into insoluble matrices. Fn exerts important effects on cell growth and differentiation through a number of discrete functional domains. Several microorganisms are known to bind Fn. We show that, under physiological conditions, HIV-1 gp120 and gp160 are capable of binding plasma and cellular Fn as well as laminin and vitronectin. Experiments were set up to analyze in detail the binding of HIV gp120 and gp160 to Fn. The gp120 and gp160 specifically recognize the C-terminal heparin-binding domain of Fn (Fn-CTHBD) with a calculated K_D of 2.8×10^{-7} M for gp160. Binding of gp160 to Fn-CTHBD is a saturable and specific process that is blocked by antibodies to Fn-CTHBD and by heparin and is inhibited to a minor extent by heparan sulfate and dextran sulfate. These observations suggest that gp120/160 specifically recognize the III_{15} repeat within Fn-CTHBD. Intact Fn and Fn-CTHBD strongly inhibit the interaction of gp120/160 with soluble CD4 and, under low serum conditions, are capable of neutralizing the infectivity of HIV-1 for CD4-positive T cells. Thus, Fn that is present in plasma and mucinous secretions may well affect HIV infectivity and virus distribution in vivo. ***J. Med. Virol. 54:44–53, 1998.***

KEY WORDS: HIV; envelope glycoproteins; gp120; gp160; fibronectin; laminin; extracellular matrix proteins

INTRODUCTION

The extracellular matrix (ECM) is a biologically active network made up of secreted components that has profound effects on cellular physiology. Cell migration, proliferation, and differentiation are examples of biological processes influenced by composition and organization of ECM. The interaction of eukaryotic cells with ECM components is mediated through specific receptors termed integrins, a family of structurally related heterodimers. Integrins allow binding of cells to collagens, proteoglycans, and a variety of glycoproteins such as fibronectin and laminin [Ruoslahti, 1991].

Fibronectins (Fn) are conserved glycoproteins of approximately 440 kDa that are composed of two almost identical chains; they are present in fibrillar form in the ECM of the loose connective tissue, in basement membranes, and in pericellular spaces [Ruoslahti, 1991]. Fn are also present in body fluids at substantial levels [Ylatupa et al., 1995]: plasma (approximately 300 μg/ml), saliva and tears (2–100 μg/ml), cerebrospinal fluid (2–8 μg/ml), and cervicovaginal secretions (>50 μg/ml). A number of discrete functional domains of Fn are capable of independent binding to a variety of ligands. In addition to cells, Fn binds to fibrin, collagens, heparin, and proteoglycans [Hynes, 1990]. The cell surface integrin $\alpha_5\beta_1$ mediates direct binding of cells to Fn via the sequence Arg-Gly-Asp-Ser (RGDS) located in the cell-binding domain. The heparin-binding domain in the C-terminal half of Fn represents the major site required for interaction of Fn with hep-

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arin. It is composed of type III repeats 14, 15, and 16. Repeat III_{15} accounts for nearly all the heparin-binding activity of this domain [Bober-Barkalow and Schwarzbauer, 1991].

In addition to structural functions, Fn play important roles in the inflammatory response [Lloyd et al., 1996] and in the defense against microorganisms [Hynes, 1990], for instance, by promoting phagocytosis [Abou-Zeid et al., 1991; Flock et al., 1996; Yan et al., 1996]. Fn bind to a variety of bacteria, fungi, and viruses [Budkowska et al., 1995; House-Pompeo et al., 1996; Murakami et al., 1996; Penalver et al., 1996; Speziale et al., 1996] and appears to play a role in modulating the interaction of infectious agents with host tissues.

The human immunodeficiency virus type 1 (HIV-1) is a polytropic agent that is widely distributed in different tissues and in virtually all body fluids. Spread of HIV occurs through many different pathways [Levy, 1994; Conaldi et al., 1995]. Binding of HIV to bodily components other than specific cell receptors may undoubtedly influence virus infectivity and the spread of virus-infected cells into tissues. For instance, it is known that the C1q complement fraction [Su and Boackle, 1991] and the secretory leukocyte protease inhibitor [McNeely et al., 1995] that are present in human saliva bind to HIV-1. These components show antiviral activity in vitro. Other unidentified salivary components may contribute to the infrequent oral transmission of this agent [Fox et al., 1988].

Because the nonparenteral transmission of virus is becoming highly prevalent [Soto-Ramirez et al., 1996] and it is known that HIV infects a wide range of epithelial cells (e.g., vaginal, intestinal, mammary) [Furuta et al., 1995; Toniolo et al., 1995; Yahi et al., 1996], we investigated the interaction of HIV-1 with major ECM proteins. In the present study, we show that a 30-kDa domain of Fn binds to HIV-1 envelope glycoproteins and that, in vitro, it may counteract virus infectivity.

MATERIALS AND METHODS

Matrix Proteins, Fn Fragments, Viral Glycoproteins, and Protein Labeling

Fn were purified from human plasma [Vuento and Vaheri, 1979]. Thermolysin-generated Fn fragments (i.e., the N-terminal domain, the collagen-binding peptide and the fragment encompassing the RGD-containing cell-binding site) were isolated as described elsewhere [Borsi et al., 1986]. The DNA/heparin-binding domain, containing the type III repeats III_4, III_5, and III_6, and the C-terminal heparin-binding domain (CTHBD), containing repeats III_{14}, III_{15}, and III_{16}, were purified from bovine plasma Fn as reported elsewhere [Skorstengaard et al., 1986]. Purity of isolated fragments was assessed as reported elsewhere [Bozzini et al., 1992; Speziale et al., 1996]. In addition, the cell-binding domain (110 kDa) and the CTHBD (30 kDa) of human Fn were obtained from Calbiochem (La Jolla, CA). The C- terminal fragment of Fn, containing the second fibrin-binding site, was isolated as described elsewhere [Garcia Pardo et al., 1985]. Highly purified porcine heparins differing in different sulfatation grade and sulfate position (types G794, G1114A, G1199, G1255) were a gift of Dr. G. Torri (Istituto Ronzoni, Milan, Italy). Heparan sulfate, dextran sulfate, bovine serum albumin (BSA), fetuin, α1-acid glycoprotein, fibrinogen, and laminin were obtained from Sigma (St. Louis, MO). To remove Fn contaminants, fibrinogen was adsorbed on gelatin-Sepharose before use. Recombinant HIV-1_{IIIB} proteins (p24, gp41 gp120, gp160) and human CD4 (sCD4) produced in the baculovirus system were obtained from Intracel Co. (Cambridge, MA). Only gp160 and sCD4 were in a water-soluble form. $Na^{125}I$ (15 mCi/μg) was purchased from Amersham (Milan, Italy). HIV-1 glycoproteins were labeled by the Iodo-Gen method (Pierce, Rockford, IL) following manufacture's instructions. The estimated specific activity of radiolabeled ligands was approximately $0.5–3 \times 10^6$ cpm/μg. To confirm the results obtained with radiolabeled gp120/160, experiments were also carried out with gp120 and gp160 that had been labeled with biotin by Intracel Co. Unless otherwise specified, chemicals, immunochemicals, and tissue culture reagents were obtained from Sigma, and tissue culture vessels were obtained from Falcon (Becton-Dickinson, San Jose, CA).

Electrophoresis and Western Blotting

Electrophoresis in 10% SDS-polyacrylamide gel was performed according to Blobel and Dobberstein [1975]; blotting was performed according to the method of Towbin et al. [1979]. Proteins were electrotransferred for 2 hr at 200 mA onto nitrocellulose membrane (Sartorius, Göttingen, Germany). The membranes were incubated with 5% BSA in phosphate buffered saline (PBS; 140 mM NaCl, 10 mM phosphate, pH 7.4) for 1 hr at 22°C and then probed overnight with 5×10^5 cpm of ^{125}I-labeled gp120 or gp160 containing 0.1% BSA and 0.1% Tween 80. The membranes were washed extensively with 0.1% Tween 80 in PBS, dried, and exposed at −70°C using X-Omat AR films (Eastman Kodak, Rochester, NY). Purity of different Fn fragments and HIV proteins was evaluated by SDS-PAGE under both reducing and nonreducing conditions. After staining with Coomassie Blue, each preparation produced essentially a single band of the expected molecular mass (Fig. 3, left panel); the estimated purity of all protein preparations was >90%.

Solid Phase Binding Assay

Microtiter wells (PETG assay strips; Costar, Badhoevedorp, The Netherlands) were coated overnight with test proteins in 50 mM Na_2CO_3, pH 9.5 (0.8 μg/well in 80μl) at 4°C [Bozzini et al., 1992]. Experiments were set up to measure the binding of HIV-1 proteins to immobilized ECM proteins. Control wells were coated with BSA (1 μg/well). After washing with PBS containing 0.1% Tween 20 (PBS-T) and blocking with 2% BSA

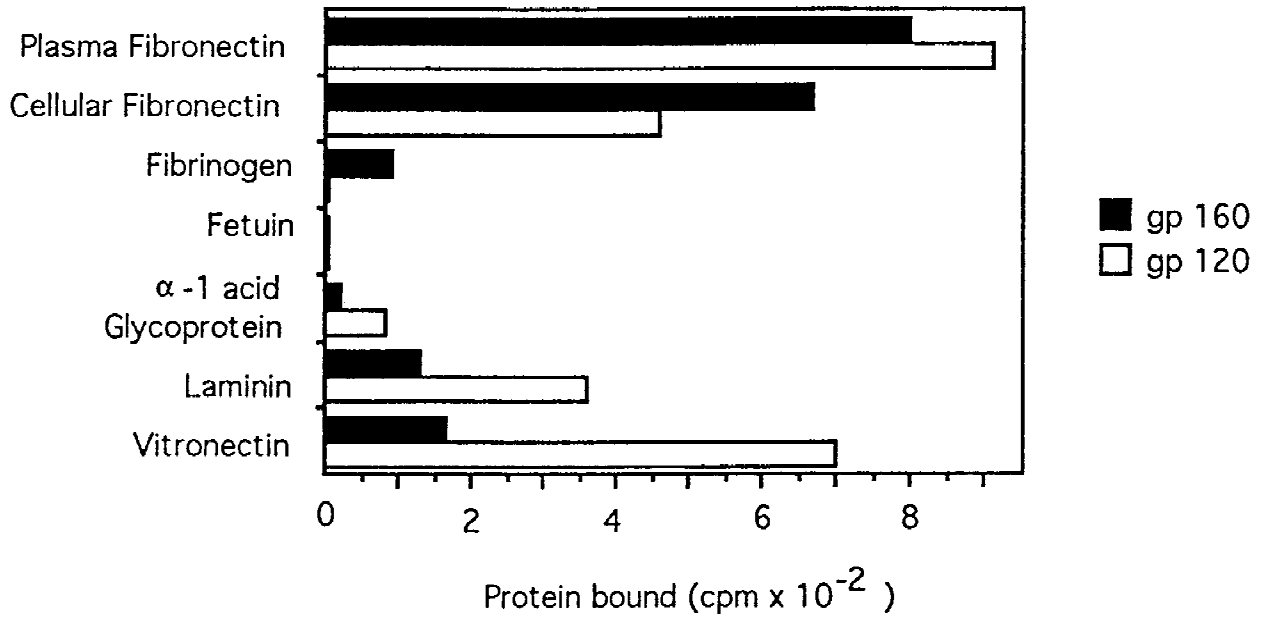

Fig. 1. Binding of ^{125}I-labeled gp120 and gp160 to immobilized extracellular matrix proteins. Microtiter wells coated with the indicated proteins (0.8 μg/well) or BSA were incubated with 1 × 10^5 cpm of ^{125}I-labeled gp160 (■) or ^{125}I-gp120 (□) in 80 μl of PBS for 2 hr at 37°C. After washing, the amount of specifically bound radiolabel was determined. Results are expressed as the mean value of duplicate wells.

in PBS for 1 hr at 22°C, the plates were rinsed with PBS-T and used for the appropriate assay. Binding assays were carried out in duplicate by using PBS-T and BSA (1 mg/ml; PBS-T-BSA). The plates were incubated with the indicated amounts of radiolabeled ligand for the indicated times at 37°C. After extensive washing with PBS-T-BSA, the amount of bound radioactivity was measured in a gamma-counter. Binding of radiolabeled p24, gp41, gp120, and gp160 to BSA-oated control wells was consistently <150 cpm/well. Results are always expressed as specific binding, i.e., as cpm/well obtained by subtracting from each test value the counts obtained in BSA-coated wells (mean of ⩾3 wells). Preliminary experiments had shown that radiolabeled p24 did not bind to the analyzed ECM proteins and that binding of gp41 was extremely low. Consequently, subsequent work was focused on the ability of gp120 and gp160 to bind ECM proteins.

Antibody Production and Isolation

Antiserum reacting with purified Fn-CTHBD was raised in mice immunized by repeated intraperitoneal injections. Serum IgG were purified on protein A-Sepharose columns (Pharmacia, Uppsala, Sweden) as recommended by the manufacturer. Rabbit IgG antibodies and mouse monoclonal antibodies to HIV-1 gp120 and gp160 were obtained from Intracel Co.

Effect of Fn on Virus Infectivity and Binding of gp120/160 to CD4

The IIIB lymphotropic strain of HIV-1 was obtained from the supernatant of chronically infected H9 cells grown in Hepes-buffered RPMI-1640 medium supplemented with 10% heat-inactivated fetal bovine serum (FBS). Clarified virus aliquots were stored at −80°C. After thawing, titers ranged between $10^{4.5}$ and $10^{5.5}$ syncytium-forming units/ml (SFU/ml) as measured in a microassay on the C8166 T lymphoblastoid cell line [Dolei et al., 1992]. The effect of ECM proteins on HIV infectivity was analyzed by mixing 90-μl aliquots of virus (approximately 100 SFU) in serum-free medium (Hepes-buffered RPMI-1640 plus 100 μg/ml BSA) with various amounts of test proteins in a final volume of 100 μl. Incubation was carried out in 96-well plates at 37°C for 2 hr. Each well then received 1.5 × 10^5 C8166 cells in 100 μl of medium enriched with 4% FBS. After 4–5 days of incubation, syncytia were counted by inverted microscopy in each well. The experiments were run in triplicate.

The interaction of radiolabeled gp120/160 with sCD4 was studied in a solid-phase system in which 96-well plates were coated with sCD4 (0.5 μg/well in 80 μl). In competition assays, increasing amounts of intact Fn or its purified domains were first added to each well in 100 μl PBS-BSA, and, immediately thereafter, 1 × 10^5 cpm of ^{125}I-gp160 or ^{125}I-gp120 in 25 μl were added to each well. After 2 hr incubation at 37°C, the wells were washed and counted in a gamma-counter.

RESULTS

Binding of gp120/160 to Immobilized ECM Proteins

Microtiter wells were coated with either plasma or cellular Fn, fibrinogen, fetuin, α1-acid glycoprotein, laminin, or vitronectin and probed with radiolabeled gp120 or gp160 (Fig. 1). Under these conditions, both gp120 and gp160 ligands bound to cellular and plasma Fn and to laminin and vitronectin. The kinetics of ra-

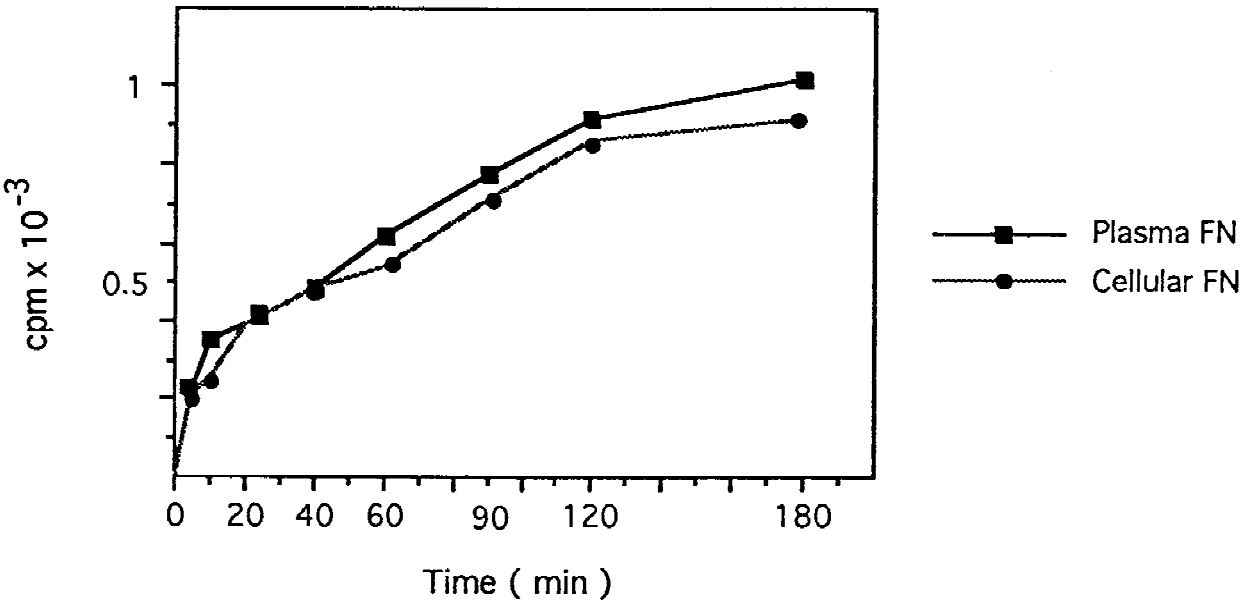

Fig. 2. Kinetics of gp160 binding to immobilized fibronectin. ^{125}I-gp160 (1×10^5 cpm) was incubated with immobilized plasma (■) or cellular (●) Fn, and the amount of bound ligand was evaluated over a 3-hr period. At the indicated times, the wells were washed and the amount of specifically bound radiolabel was determined. Data are expressed as the mean value of duplicate wells.

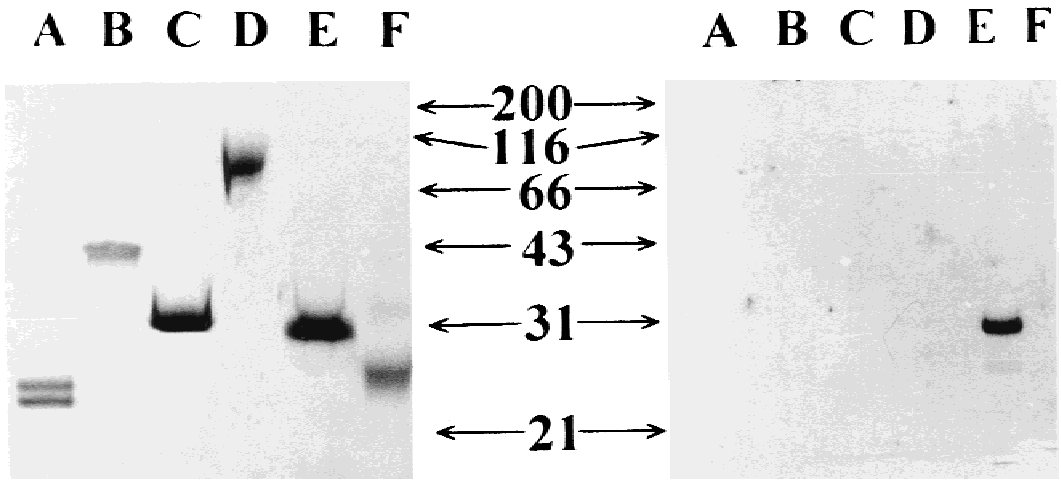

Fig. 3. Binding of ^{125}I-labeled gp160 to Fn fragments electroblotted onto nitrocellulose filter. Fn fragments were separated by SDS/PAGE (10%) under nonreducing conditions. Proteins were either stained with Coomassie Blue (**left**) or electroblotted onto nitrocellulose paper and probed with ^{125}I-labeled gp160 (**right**). **Lane A:** N-terminal fragment. **Lane B:** Collagen-binding domain. **Lane C:** DNA/heparin-binding domain. **Lane D:** Cell-binding domain. **Lane E:** C-terminal heparin-binding domain. **Lane F:** C-terminal domain. Numbers and arrows in the middle of the figure indicate molecular mass (kDa) and migration of marker proteins.

diolabeled gp160 binding to plasma and cellular Fn was examined in a solid-phase assay (Fig. 2). Binding of gp160 was a time-dependent process that reached maximal levels after 3 hr of incubation. Preliminary experiments showed that p24 did not bind to ECM proteins and that binding of gp41 was minimal (not shown). In the course of these experiments, we also realized that gp120 and gp160 bound to Fn to approximately the same extent; radiolabeled gp160, however, provided more consistent results. The use of biotin-labeled gp120 and gp160 produced results equivalent to those obtained with radiolabeled probes (protein labeling by Intracel). The higher solubility of gp160 may be responsible for this phenomenon. For this reason, only results of gp160 binding are reported in most cases.

Binding Sites for gp120/160 in Fn

To identify the sites in Fn involved in binding to gp120/160, microtiter wells were coated with purified Fn fragments. Test fragments included the N-terminal domain (28 kDa), the collagen-binding domain (40 kDa), the DNA/heparin binding domain (30 kDa), the fragment containing the RGD cell-binding sequences

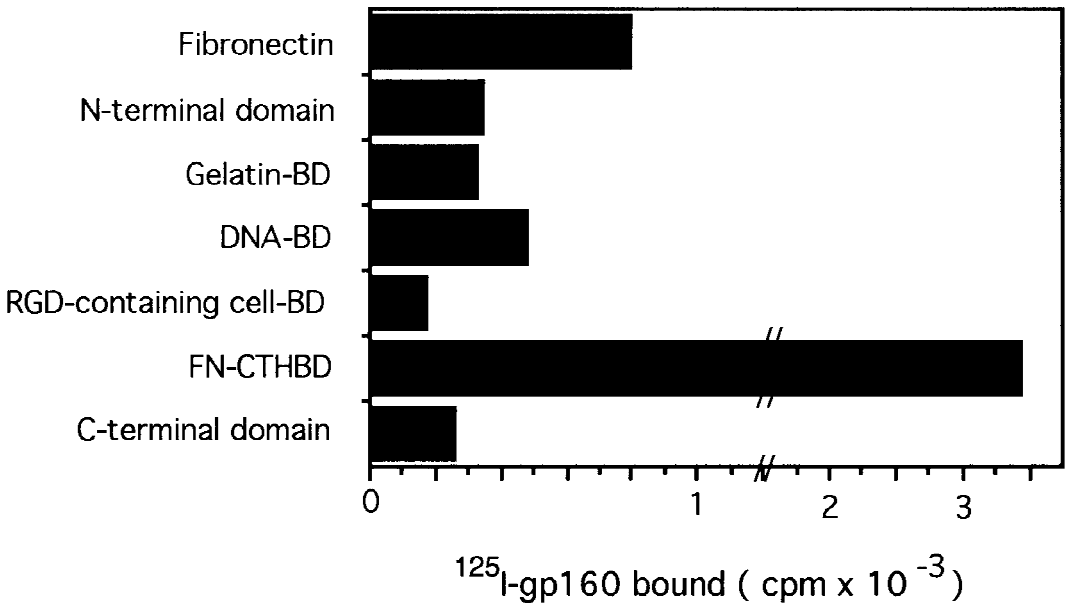

Fig. 4. Binding of ^{125}I-labeled gp160 to Fn fragments adsorbed to microtiter wells. Microtiter wells coated with 0.8 μg of either Fn or its fragments were incubated with 1×10^5 cpm of ^{125}I-labeled gp160. After 2 hr, the wells were washed and the amount of specifically bound radiolabel was determined. Bars represent the mean values of duplicate wells.

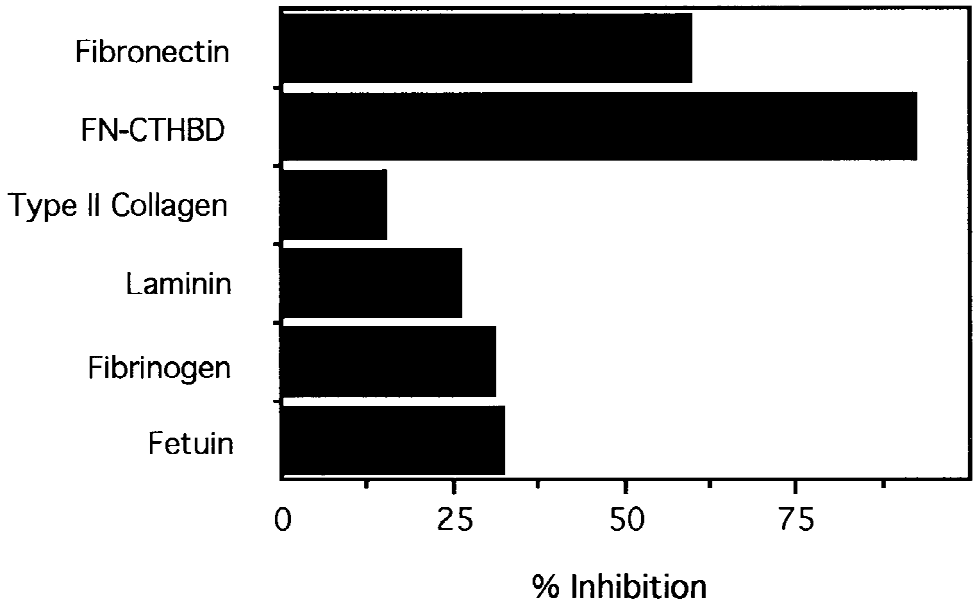

Fig. 5. Specificity of ^{125}I-labeled gp160 binding to the C-terminal heparin-binding domain of Fn. ^{125}I-labeled gp160 (1×10^5) was incubated for 2 hr at 37°C with Fn-CTHBD (0.8 μg/well) in the presence of 10 μg of the indicated competitor proteins. The amount of bound ligand is given as a percentage of the radioactivity specifically bound in the absence of competing proteins (mean of duplicate determinations; specific binding in the absence of competing proteins was 1,638 ± 50 cpm/well, mean ± SD; n = 4).

(110 kDa), the CTHBD (30 kDa), and the C-terminal fibrin-binding domain (31 kDa). Figure 3 (left panel) shows the electrophoretic pattern of Fn fragments. As shown in Figure 4, substantial binding of ^{125}I-labeled gp160 occurred only in wells coated with either native Fn or its CTHBD. Other Fn fragments did not interact with the ligand to a substantial extent. Binding of gp160 to the CTHBD was higher than that to the intact Fn molecule. Sterical freedom or a favorable conformational change may be responsible for the enhanced binding of this ligand to the fragment.

Complex formation of gp120/160 with Fn fragments was also studied by Western blot analysis (Fig. 3, right panel). Fn fragments were run on SDS/PAGE under nonreducing conditions and transferred to nitrocellulose membranes. Blots were then incubated with ^{125}I-labeled gp120/160, rinsed, and autoradiographed. As expected, gp160 recognized almost exclusively the 30-

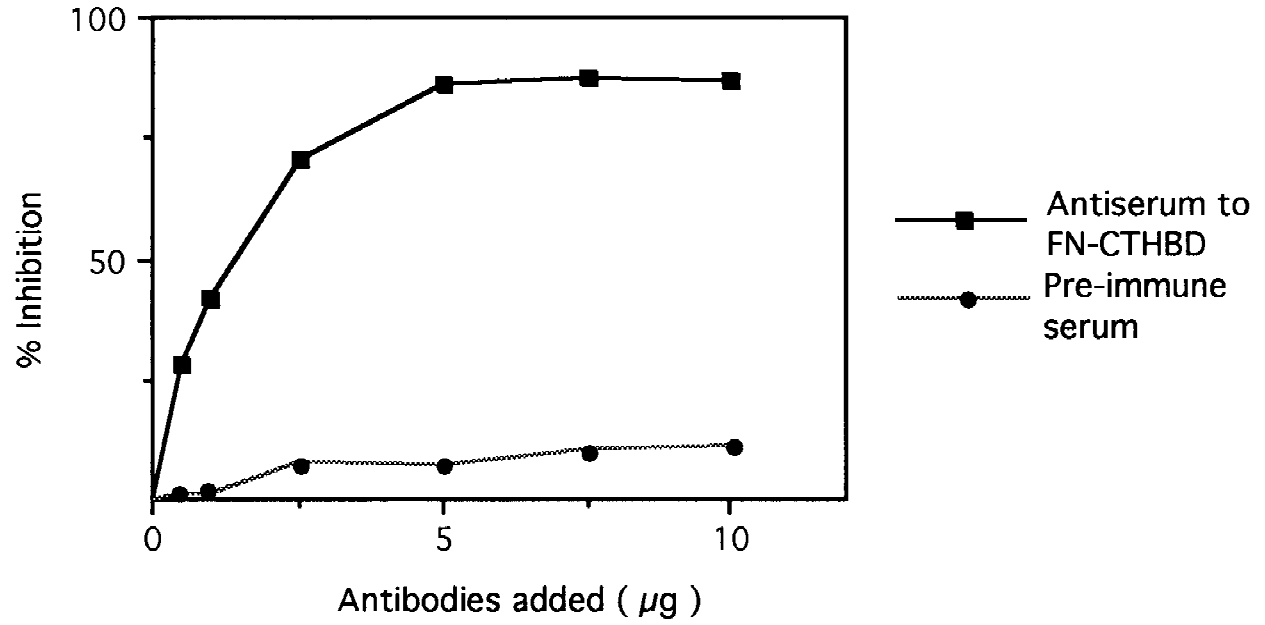

Fig. 6. Effect of an antibody directed to Fn-CTHBD on binding of gp160 to Fn-CTHBD. Microtiter wells coated with Fn-CTHBD (0.8 μg/well) were incubated for 2 hr at 37°C with 1 × 10^5 cpm of ^{125}I-labeled gp160 and 0-10 μg of IgG purified from either preimmune (●) or immune (■) serum against Fn-CTHBD. The amount of bound ligand is given as a percentage of the radioactivity specifically bound in the absence of IgG (mean of duplicate determinations; specific binding in the absence of competing proteins was 2,174 ± 123 cpm/well, mean ± SD; n = 4).

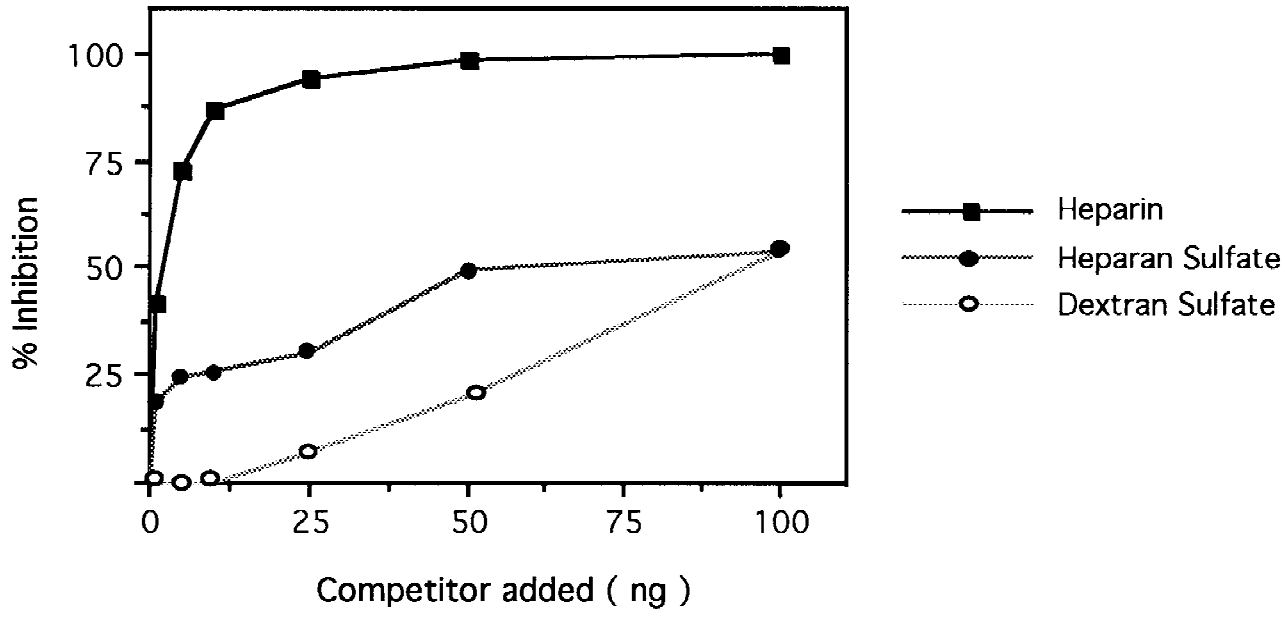

Fig. 7. Inhibitory activity of heparin on the binding of gp160 to Fn-CTHBD. ^{125}I-labeled gp160 (1 × 10^5 cpm) was incubated for 2 hr at 37°C with wells coated with 0.8 μg of Fn-CTHBD in the presence of increasing concentrations of heparin G794 (■), heparan sulfate (●), and dextran sulfate (○). After extensive washing, bound radiolabel was measured. The amount of bound ligand is given as a percentage of the radioactivity specifically bound in the absence of competing substances (mean of duplicate determinations; specific binding in the absence of competitors was 1554 ± 95 cpm/well, mean ± SD; n = 4).

kDa Fn-CTHBD, whereas gp120 recognized both the 30-kDa CTHBD domain and the 30-kDa DNA/heparin-binding domain of Fn (data not shown).

Specificity of the Interaction Between gp160 and Fn-CTHBD

Specificity of the above interaction was assessed by analyzing the binding of ^{125}I-labeled gp160 to wells coated with Fn-CTHBD in the presence of unlabeled ECM proteins. As expected, binding of gp160 was substantially inhibited by intact Fn and by isolated Fn-CTHBD. By contrast, collagen type II, laminin, fibrinogen, and fetuin interfered weakly with the binding (Fig. 5). Mouse polyclonal antibodies raised against Fn-CTHBD were also tested for their ability to inhibit the binding of gp160 to Fn fragments. The results indicate

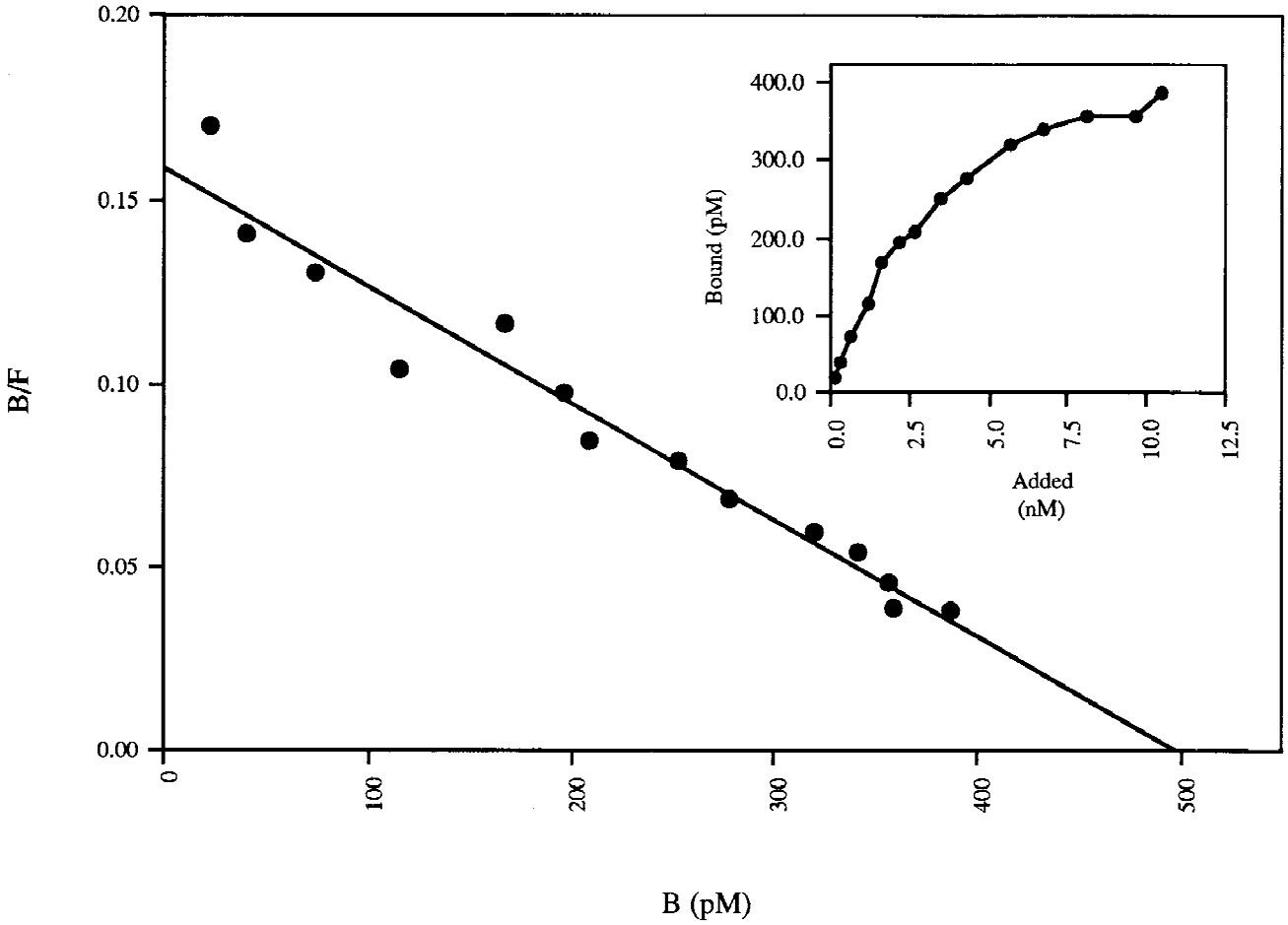

Fig. 8. Scatchard plot analysis of gp160 binding to solid phase-adsorbed Fn-CTHBD. Microtiter wells coated with 0.8 μg of Fn-CTHBD were incubated with ^{125}I-gp160 (specific activity 10^6 cpm/5 μg). After 2 hr incubation at 37°C, wells were washed and the amount of specifically bound radioactivity was determined. Binding data for kinetic analysis were calculated by a nonweighted linear regression computer program. The **inset** shows the saturation binding isotherm of gp160 to the fragment. B/F, bound over free gp160.

that IgG specific for the CTHBD effectively inhibited the interaction, whereas IgG from preimmune serum did not (Fig. 6).

The Fn-CTHBD consists of type III repeats 14, 15, and 16, each with 90 amino acids. The major heparin-binding site resides in repeat III_{15} [Bober-Barkalow and Schwarzbauer, 1991]. To investigate whether gp160 and heparin share the same binding site(s), microtiter wells were coated with the Fn-CTHBD and incubated with ^{125}I-labeled gp160 in the presence of different concentrations of heparins. Heparins differing in sulfatation grade and in sulfate position were tested. Different heparin types inhibited by over 95% the binding of gp160 to CTHBD at approximately 20 ng/ml (data in Fig. 7 refer to heparin G794; results of other heparin types are not shown), whereas heparan sulfate and in particular dextran sulfate were much less effective (Fig. 7). Thus, gp160 may interact mainly with repeat III_{15}, i.e., with the major site recognized by heparin [Bober-Barkalow and Schwarzbauer, 1991; Walker and Gallagher, 1996].

Binding of gp160 to Fn-CTHBD was found to be a saturable process. Radiolabeled gp160 was mixed with unlabeled gp160 to give a specific activity of 10^6 cpm/5 μg. Increasing amounts of the mixture were added to fragment-coated wells. Under equilibrium conditions in the solid-phase assay, Scatchard analysis of gp160 binding to the Fn peptide fitted a straight line, suggesting the presence of one class of binding sites for gp160. The calculated value of the dissociation constant of the complex was 2.8×10^{-7} M (Fig. 8). Binding was not influenced by divalent cations (1 and 4 mM Ca^{2+} and Mg^{2+}; data not shown). Comparable results were obtained with gp120, but the process was not fully saturable; it was therefore not possible to measure the dissociation constant (data not shown).

Effects of Fn and Fn-CTHBD on HIV Infectivity and on Binding of gp120 to CD4

Increasing amounts of Fn or Fn-CTHBD were incubated under serum-free conditions with approximately 100 SFU of virus. After 2 hr incubation, T-lymphoblastoid C8166 cells were added, and virus-induced syncytia were counted after a 5-day incubation. As shown in Table I, syncytia formation, which reflects the amount of infectious particles [Dolei et al., 1992], was

TABLE I. Effect of Fn and Its C-Terminal Heparin-Binding Domain on HIV-1 Infectivity and on the Binding of gp120 to Solid-Phase Adsorbed sCD4

	Syncytia formation[b]		Binding of gp120 to sCD4[c]	
Protein, μg/ml[a]	SFU/ well	% of inhibition	cpm/ well	% of inhibition
BSA, 100	76 ± 11	0	n.d.	—
BSA, 1,000	n.d.	—	4,501	0
Fibronectin, 3.7	78 ± 21	0	2,385	47
Fibronectin, 7.5	59 ± 10	19	1,980	56
Fibronectin, 15	45 ± 21	39	1,731	62
Fibronectin, 30	33 ± 18	55	1,350	70
Fibronectin, 60	34 ± 13	54	927	79
C-CTHBD, 3.7	67 ± 12	8	n.d.	
C-CTHBD, 7.5	47 ± 16	36	n.d.	
C-CTHBD, 15	34 ± 13	44	n.d.	
C-CTHBD, 30	26 ± 9	64	n.d.	
C-CTHBD, 60	20 ± 12	73	n.d.	
C-CTHBD, 0.6	n.d.		2,302	49
C-CTHBD, 1.2	n.d.		1,601	64
C-CTHBD, 2.5	n.d.		1,456	68
C-CTHBD, 5	n.d.		1,264	72
C-CTHBD, 10	n.d.		1,158	74

[a]Concentration of test proteins. In the study of gp120/sCD4 interaction, approximately equimolar amounts in Fn and its C-CTHBD were used. To this end, the dose of C-CTHBD was reduced by a factor of 6 as compared with that of intact Fn (e.g., 60 μg/ml of Fn and 10 μg/ml of C-CTHBD represented the 330-nM concentration).
[b]Syncytium-forming units/well on the T lymphoblastoid C8166 cell line (mean ± SD of two experiments run in triplicate). The indicated amounts of test proteins were incubated for 2 hr with 75 SFU of virus.
[c]Specific binding of radiolabeled gp120 to wells coated with soluble CD4.

inhibited in a dose-dependent manner by both Fn and its CTHBD. To gain some insight into the possible mechanism(s) responsible for the reduction of virus yields, we investigated the influence of these proteins on the binding of gp120 to wells coated with sCD4. Both Fn and Fn-CTHBD inhibited gp120 binding to CD4 in a dose-dependent manner. Over 70% inhibition of binding occurred at doses of 30 μg/ml (intact Fn) or 5 μg/ml (CTHBD; Table I). Equivalent results were obtained when the gp120/CD4 interaction was studied (Table I). Thus, reduction of virus infectivity by Fn may be due to perturbation of virus–CD4 interaction.

DISCUSSION

Binding of ECM proteins to viral particles or viral components has been observed in a number of studies. Orthomyxoviruses and paramyxoviruses have surface hemagglutinins that mediate their binding to the sialic acid-containing glycoproteins of both the cell surface and ECM. Binding of influenza A and mumps viruses to Fn appears to involve sialic acid residues [Julkunen et al., 1983]. Binding of hepatitis A virus to Fn was suggested to favor the transmission of infection through Fn-containing blood products [Seelig et al., 1984]. Binding of hepatitis B virus pre-S2 region to Fn present on liver sinusoids appears to facilitate virus uptake by hepatocytes [Budkowska et al., 1995]. Among retroviruses, the gag polyprotein of Abelson murine leukemia virus binds laminin [Boss et al., 1981], and Rous sarcoma virus remains associated with Fn present in the ECM produced by infected chick fibroblasts [Stanislawski, 1983].

Binding of cells or virus particles to ECM proteins is associated with a variety of biological effects. The efficiency of infection by retroviral vectors is greatly enhanced when hematopoietic cells are attached to the RGD-containing domain of Fn [Moritz et al., 1994]. Binding of HIV-1 tat protein to ECM helps retain a positive regulatory signal in the proximity of infected cells [Albini et al., 1995]. In thymic epithelial cells, tat production results in the inadequate expression of membrane-associated Fn and in alterations of thymocyte development [Maroder et al., 1996], which is apparently mediated by binding of the RGD sequence of tat to the cellular Fn receptor. It is also known that the salivary secretory leukocyte protease inhibitor (SLPI) is associated with the surface of lymphoid cells: binding of HIV-1 to SLPI prevents virus entry into target cells and neutralizes virus infectivity [McNeely et al., 1995].

Our results show that binding of gp160 to Fn-CTHBD is a saturable process characterized by a physiologically significant affinity constant ($K_D = 2.8 \times 10^{-7}$ M). In our hands, binding of gp120 to Fn-CTHBD was not a saturable process, thus the affinity constant could not be measured. The difference may be due to the higher solubility of gp160 as compared with that of gp120. Binding of both gp120 and gp160 occurs in the normal pH range and does not require divalent cations. Binding to Fn-CTHBD is specific because it is not inhibited by excess amounts of unrelated proteins and is blocked only by specific-IgG raised against this particular Fn domain. The interaction between gp120/160 and Fn is completely inhibited by heparins and only reduced by heparan sulfate. This observation suggests that gp120/160 may recognize the III_{15} repeat within Fn-CTHBD [Bober-Barkalow and Schwarzbauer, 1991; Walker and Gallagher, 1996]. The data, however, do not exclude that adjacent repeats contribute to stabilizing the binding.

In addition to these observations, Fn and its CTHBD appeared to neutralize HIV infectivity for cultured T lymphoblastoid cells. Viral neutralization by plasma Fn also has been reported in the case of human cytomegalovirus [Agbanyo and Wasi, 1994]. We documented that, under low serum conditions, HIV-1 is neutralized by Fn and Fn-CTHBD at concentrations far below those found in normal plasma (approximately 300 μg/ml) and in mucinous secretions (≥50 μg/ml). Experiments were performed in the 0.6–60 μg/ml concentration range. Because Fn and Fn-CTHBD appear to compete with similar kinetics with the binding of gp120 to CD4, neutralization of virus infectivity by Fn and Fn-CTHBD may well be due to interference with the attachment of HIV to the cell surface.

The biological implications of the interaction between HIV envelope glycoproteins and Fn are appealing. It is tempting to speculate that, as observed in vitro, HIV binding to Fn present in mucinous secretions may protect mucosal epithelial cells from infec-

tion. Thus, Fn might well contribute to the reduction of HIV transmission through the vaginal and oral routes [Su and Boackle, 1991; McNeely et al., 1995]. However, it is also possible that cell-bound Fn may enhance virus trapping in proximity of the plasma membrane, thus favoring its entry into cells.

Due to the multifaceted in vivo activities of Fn, binding of gp120/160 to Fn can play several additional roles. For instance, because Fn binds the C1q complement component [Ingham et al., 1985], attachment of HIV to Fn could lead to C1q fixation. This event may result in either virus neutralization or virus internalization into a variety of cells bearing C1q receptors [van den Berg et al., 1995; Söelder et al., 1989].

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